Docket No. **112/002/CON1**

PATENT



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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In re Application of:

**LIGHT**, Elizabeth S.

Group Art Unit: **1655**

Examiner: **CHAKRABARTI**

Serial No.:
**09/863,125**

Attorney Docket No.: **112/002/CON1**

Filed: **5/22/01**

For: **Method of Detecting Single Gene Copies *in situ***

**Response and Amendment under 37 CFR 1.111**

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

This Response and Amendment is filed in response to the Office Action dated 4/12/2002. A Petition for an extension of time to respond is hereby included. Please amend the application as follows:

**In the claims:**

Please cancel claims 20-24 as being drawn to a non-elected invention. Applicants specifically reserve the right to re-file them in a divisional filing.

Please cancel claims 25-26, and replace them with the following new claims 27-30:

27. A method of visually detecting a single copy of the Her-2/neu gene in chromosomal DNA in an intact cell using brightfield microscopy, comprising:

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heating the tissue or cell sample sufficiently to dissociate the native chromosomal target strands of Her-2/neu DNA;

contacting said tissue or cell sample with a detectably-labeled nucleic acid Her-2/neu probe specific for the Her-2/neu gene under conditions that allow the re-hybridization of the labeled nucleic acid Her-2/neu probe and target strands of Her-2/neu DNA to form a target-probe duplex;

contacting the target-probe duplex with an anti-label antibody under conditions allowing the antibody to bind to the label;

contacting the anti-label antibody with an enzyme and a chromogen composition under conditions allowing the development of a visually detectable chromogen substrate signal at each target-probe duplex separate and distinct from the chromogenic signals of other copies of said chromosomal target nucleic acid sequence; and

detecting the chromogenic substrate signal using brightfield microscope conditions.

28. The method of claim 27 wherein the detectably-labeled nucleic acid probe is labeled with a moiety selected from the group consisting of digoxygenin, biotin and fluorescein.

29. The method of claim 27 wherein the enzyme is selected from the group consisting of a phosphatase and a peroxidase.

30. The method of claim 27 wherein the chromogen is selected from the group consisting of NBT/BCIP, tetramethylbenzidine and diamino benzidine.

**Comments**

Claims 25 and 26 are currently pending in this application. Applicants have canceled claims 25-26 herein and substitute claims 27-30 in their place. Applicants respectfully request reconsideration of the application.

Applicant has added new claims 27-30 to more specifically claim and distinctly point out the inventive aspects of her invention. In particular, Applicant has canceled the previous method claims and substituted a more traditional method claim that denotes the individual steps of a diagnostic method. Support for new claim 27 is found throughout the specification, but particularly in Examples 1 and 2 from p. 15, line 9, through p. 17, line 18. No new matter has been introduced by this amendment.

1. Restriction Requirement.

Applicants hereby affirm the election to prosecute claims 25-26. Applicants further acknowledge that claims 20-24 are presently withdrawn from examination.

2. 35 USC 112 Rejection of claims 25-26

Claims 25-26 have been cancelled herein, mooting this basis of rejection.

3. 35 USC Section 102(b) under Singer et al.

Claims 25-26 were rejected under 35 USC 102(b) as being anticipated by Singer et al. These claims have been cancelled herein, however assuming the Examiner believes the reference still is relevant, Singer et al. is discussed. Applicants respectfully traverse the rejection, for the following reasons.

Singer et al. refer to a method for strand displacement amplification of HIV (Human Immunodeficiency Virus) *in situ* followed by detection. The goal is to detect low copy numbers of HIV *in situ* without reliance upon PCR or hybridization, as stated at column 3, lines 15+. By contrast, the present invention quantifies the number of gene copies in individual cells *in situ*. If

quantifiable at all in Singer et al, amplification of the target would give the wrong number of gene copies.

The claimed product differs from the result of the Singer et al method in several respects.

Claim 27 requires that the chromogenic product associated with one copy of the target sequence is separate from the chromogenic product associated with another copy. In short, when viewing the cell under a microscope in the present invention, one sees a cell with distinct blue-black spots inside it. The "products" are distinguishable, e.g., they do not overlap so as to destroy the ability to distinguish one copy from the other. See Figures 1-3 in the present application. By contrast, Singer et al amplify the target sequence so that thousands to millions of product spots are present which causes the entire stained portion of the cell to appear as a smear. See Figure 1A of Singer et al. The "products" in Singer et al are not "separate" from each other but rather are indistinguishable, which is outside of the scope of the present claims.

Furthermore, in Singer et al, one cannot determine the number of original copies of the target nucleic acid sequence in the cell. Singer et al amplify the target nucleic acid. During amplification, original target copy quantification information is lost.

Thus, the Singer et al composition is different from that presently claimed and the rejection should be withdrawn. It is well-known that for a claim to be anticipated, all of the elements of the invention must be shown in the prior art reference. Clearly, Singer does not teach a composition for visually distinguishing single from individual targets. Therefore, Applicant respectfully requests reconsideration of the application as amended, and requests an indication of allowance of the claims.

4. 35 USC Section 103 Rejection of claims 25-26 under Duhamel, US 6068843

Claims 25-26 were rejected under 35 USC 103(a) as being obvious by the combination of by Singer et al. and Duhamel et al. These claims have been cancelled herein, however assuming the Examiner believes the reference still is relevant, Duhamel et al. is distinguished. Applicants respectfully traverse the rejection, for the following reasons.

Duhamel et al. teach a method for the detection of *S. hyodysenteriae* in a biological sample by using a *S. hyodysenteriae*-specific oligonucleotide probe and PCR primers for measuring the presence or absence of amplified *S. hyodysenteriae*-specific sequences from a 2.3 kb HindIII restriction fragment of B204 serotype 2. Col. 4, lines 16-23. The amplification products can be detected by dot blot or Southern blot hybridization analysis. Col. 4, lines 39-45. Alternatively, the PCR products can be detected by immobilization to a bead or a multiwell plate by a probe or primer labeled with biotin, followed by hybridization with a detectably labeled probe. Col. 4, lines 47-50. Duhamel also teach that a DNA probe could be fashioned that would target 1.55kb section of the 2.3kb HindIII fragment. Col. 5, lines 23-33.

Duhamel et al. do not show chromogenic detection. Nor do they show single copy detection. Duhamel's main thrust is a PCR assay, which requires amplification of a target sequence using PCR primers. No examples are taught for using a probe to detect the sequence in situ, let alone for single copy detection.

Applicant's comments above regarding Singer et al. are repeated by reference herein. Neither reference teaches Applicant's demonstrated detection of a single copy of any gene *in situ* using chromogenic detection. Both references teach the detection of a gene sequence first by amplifying the sequence, then detecting it. Applicant respectfully suggests that the Examiner has not met his burden of establishing a *prima facie* case of obviousness, and so requests that the rejection be withdrawn on that basis.

Respectfully submitted,

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Huw R. Jones, Esq.
Attorney for Applicants
Reg. No. 33,916